Supporting Information

"ExoSensor 517: A Dual-Analyte Fluorescent Chemosensor for Visualizing Neurotransmitter Exocytosis"

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Table of Contents Page

I. Spectroscopic Studies

Solution preparations. Solutions for UV/Vis and fluorescence spectroscopy were prepared from a 1 mg/mL sensor stock solution in DMSO that was diluted to volume with buffer (50 mM bis tris propane and either 120 mM NaCl or 50 mM $Na_2S_2O_3$). DMSO was added to equal 1% of the total volume. Analyte solutions were prepared by dissolving solid analyte in the buffered sensor solution described above such that the concentration of sensor remained constant. Aromatic analyte solutions were prepared at lower concentrations due to stronger binding and tendencies toward oxidation at higher pH values. Absorption spectra were recorded on a Cary 1E spectrophotometer at 25 ºC. Fluorescence spectra were recorded on a Shimadzu RF-5301 PC spectrofluorometer at 25 ºC.

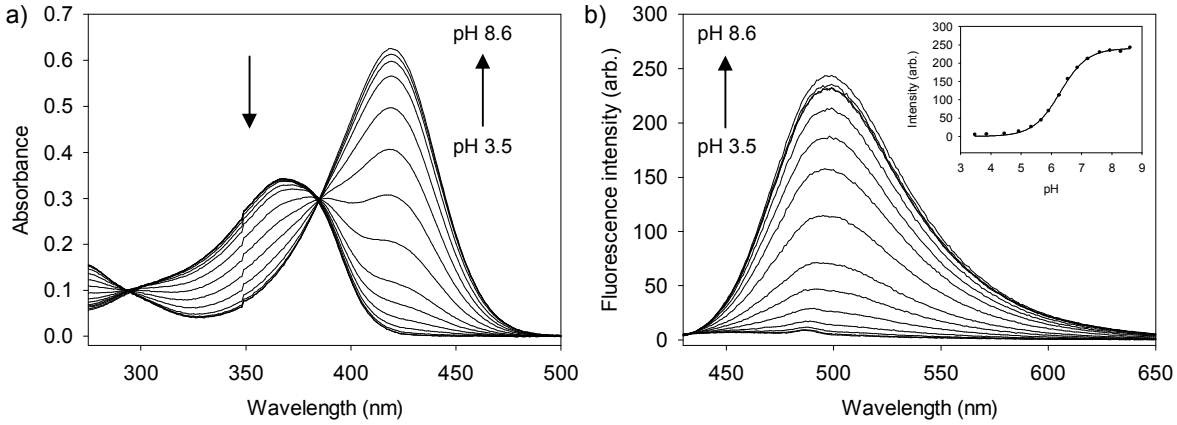


Figure S1. (a) Absorbance and (b) fluorescence pH titration of **ES517** (20 μM) in buffer (50 mM bis tris propane, 120 mM NaCl, 1% DMSO) adjusting the pH from 3.5 to 8.6. λ_{ex} = 419 nm. Inset is the fit to a pH isotherm. pK_a = 6.3.

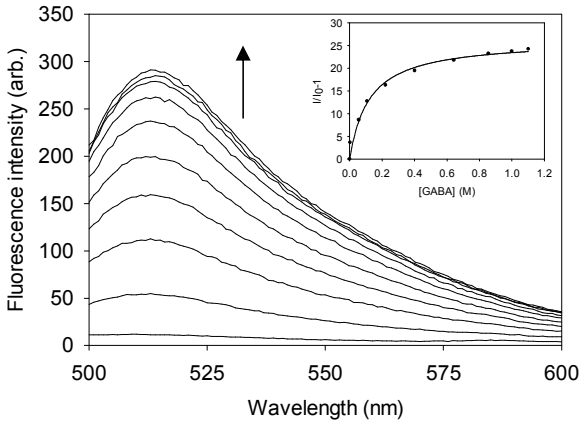


Figure S2. Fluorescence titration of **ES517** (20 μM) with 20-800 μL of 1.5 M GABA in buffer (50 mM bis tris propane, pH 7.4, 50 mM $Na_2S_2O_3$, 1% DMSO). λ_{ex} = 488 nm. Inset is the fit to a binding isotherm. λ_{em} = 514 nm.

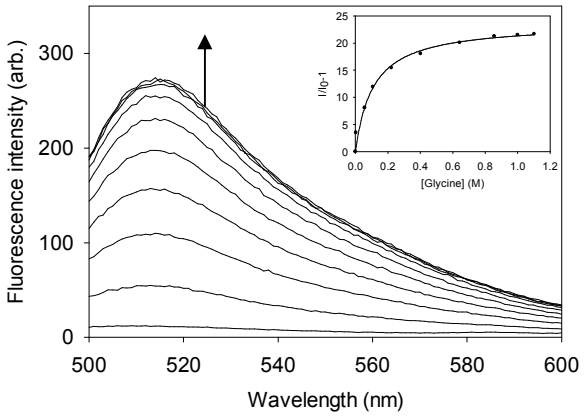


Figure S3. Fluorescence titration of **ES517** (20 μM) with 20-800 μL of 1.5 M glycine in buffer (50 mM bis tris propane, pH 7.4, 50 mM $Na_2S_2O_3$, 1% DMSO). λ_{ex} = 488 nm. Inset is the fit to a binding isotherm. λ_{em} = 515 nm.

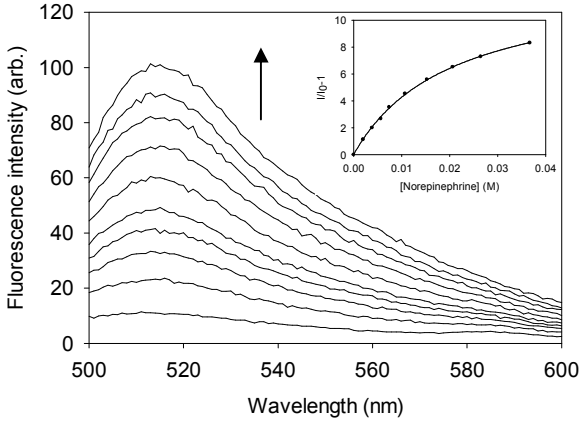


Figure S4. Fluorescence titration of **ES517** (20 μM) with 20-220 μL of 100 mM norepinephrine in buffer (50 mM bis tris propane, pH 7.4, 50 mM $Na_2S_2O_3$, 1% DMSO). λ_{ex} = 488 nm. Inset is the fit to a binding isotherm. λ_{em} = 515 nm.

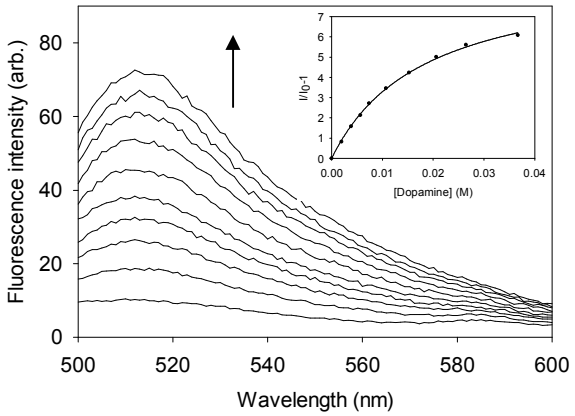


Figure S5. Fluorescence titration of **ES517** (20 μM) with 20-220 μL of 100 mM dopamine in buffer (50 mM bis tris propane, pH 7.4, 50 mM $Na_2S_2O_3$, 1% DMSO). λ_{ex} = 488 nm. Inset is the fit to a binding isotherm. λ_{em} = 513 nm.

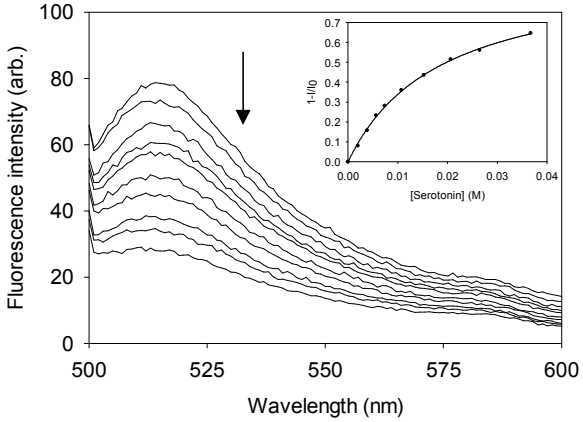


Figure S6. Fluorescence titration of **ES517** (20 μM) with 20-220 μL of 100 mM serotonin in buffer (50 mM bis tris propane, pH 7.4, 50 mM $Na_2S_2O_3$, 1% DMSO). λ_{ex} = 488 nm. Inset is the fit to a binding isotherm. λ_{em} = 514 nm.

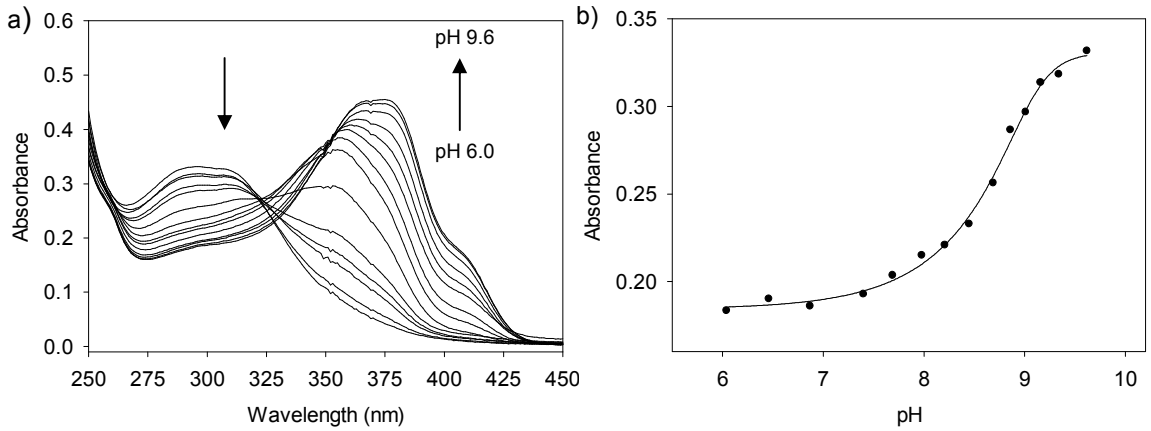
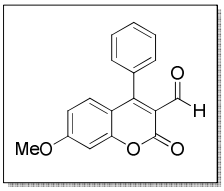


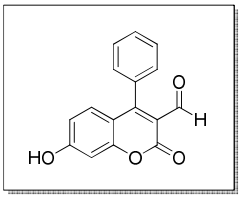
Figure S7. (a) UV/vis titration and (b) pH isotherm of compound **2** (20 μM) and 0.5 M glutamate adding aliquots of 1-8 M NaOH in buffer (50 mM bis tris propane, 120 mM NaCl). pK_a = 9.1

II. Synthetic Procedures

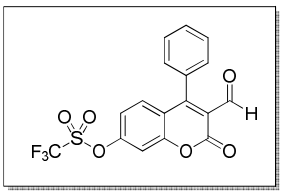
All chemicals were obtained from Aldrich, Fisher, TCI America, or Alfa Aesar and were used without further purification. Flash chromatography was performed with 32-63 μm silica gel. NMR spectra were recorded on a Bruker DRX 500. IR spectra were recorded on a Nexus 670 FT-IR E.S.P. spectrometer.



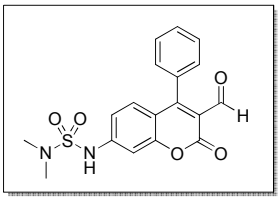
Compound 2. A mixture of compound **1** (129 mg, 0.541 mmol), $PhB(OH)_2$ (79.1 mg, 0.649 mmol), Pd_2dba_3 (24.7 mg, 0.027 mmol), SPhos (33.0 mg, 0.081 mmol), K_3PO_4(230 mg, 1.082 mmol) degassed for 15 minutes. Dry, degassed THF (3.2 mL) was added and the solution stirred at 60 °C for 12 h. The solution was filtered and the solvent removed *in vacuo*. The remaining residue was purified by chromatography (95:5 CH_2Cl_2/EtOAc) to yield compound **2** (107 mg, 71%) as a yellow solid (mp 164 °C): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.90 (s, 1H), 7.51-7.58 (m, 3H), 7.26-7.31 (m, 2H), 7.11 (d, 1H, *J* = 9.0 Hz), 6.87 (d, 1H, *J* = 2.5 Hz), 6.78 (dd, 1H, *J* = 9.2, 2.5 Hz), 3.91 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 188.2, 165.4, 161.8, 158.7, 156.9, 132.0, 130.7, 129.7, 128.7, 128.4, 115.9, 113.6, 113.3, 100.6, 56.1; IR (neat, cm^{-1}) 2846, 1756, 1720, 1614, 1581, 1532, 1374, 1296; HRMS calculated for $C_{17}H_{12}O_4Na$ ($M + Na^+$): 303.0628. Found: 303.0628.



Compound 3. A solution of compound **2** (38 mg, 0.136 mmol) in CH_2Cl_2 (2 mL) was added to a flame-dried round bottom and cooled to -78 °C. BBr_3 (0.68 mL of a 1.0 M solution in CH_2Cl_2, 0.678 mmol) was added dropwise. The reaction was warmed to room temperature over 12 h. After being quenched with cold deionized water (3 mL), the mixture was extracted with CH_2Cl_2 (10 mL x 5). The combined organic layers were dried over Na_2SO_4, and the solvent was removed *in vacuo*. The residue was purified by chromatography (7:3 hexanes/EtOAc) to yield compound **3** (21.7 mg, 60%) as a yellow solid (mp 165 °C): ^{1}H NMR (500 MHz, d-acetone) δ 9.95 (s, 1H), 9.87 (s, 1H), 7.53-7.59 (m, 3H), 7.35-7.41 (m, 2H), 7.03 (d, 1H, *J* = 7.5 Hz), 6.80-6.85 (m, 2H); ^{13}C NMR (125 MHz, d-acetone) δ 188.3, 164.7, 161.3, 159.3, 157.8, 133.9, 132.1, 129.9, 129.3, 116.5, 114.7, 113.8, 103.4; IR (neat, cm^{-1}) 3273, 1734, 16.14, 1535, 1385, 1120; HRMS calculated for $C_{16}H_{10}O_4Na$ ($M + Na^+$): 289.0471. Found: 289.0471.

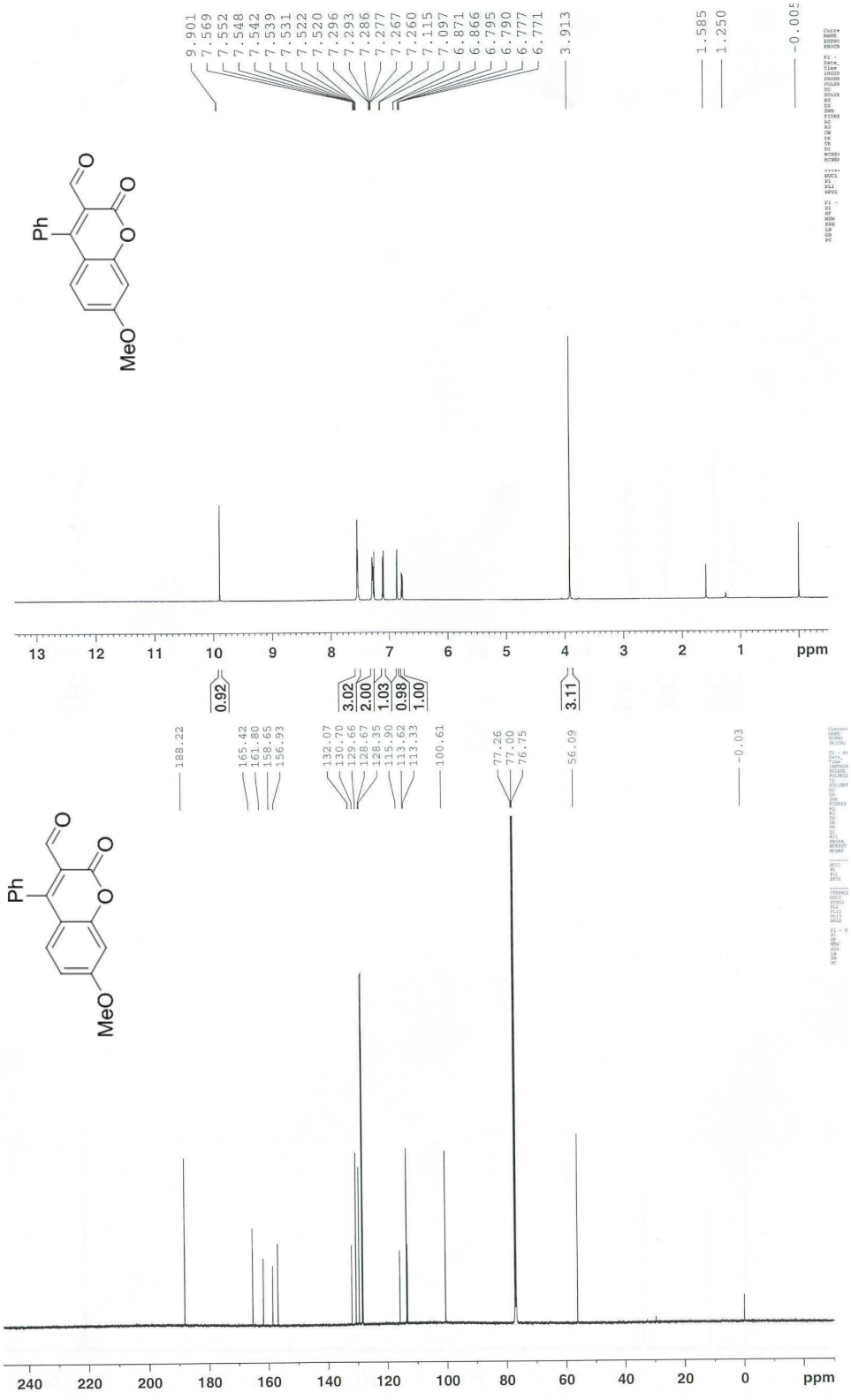


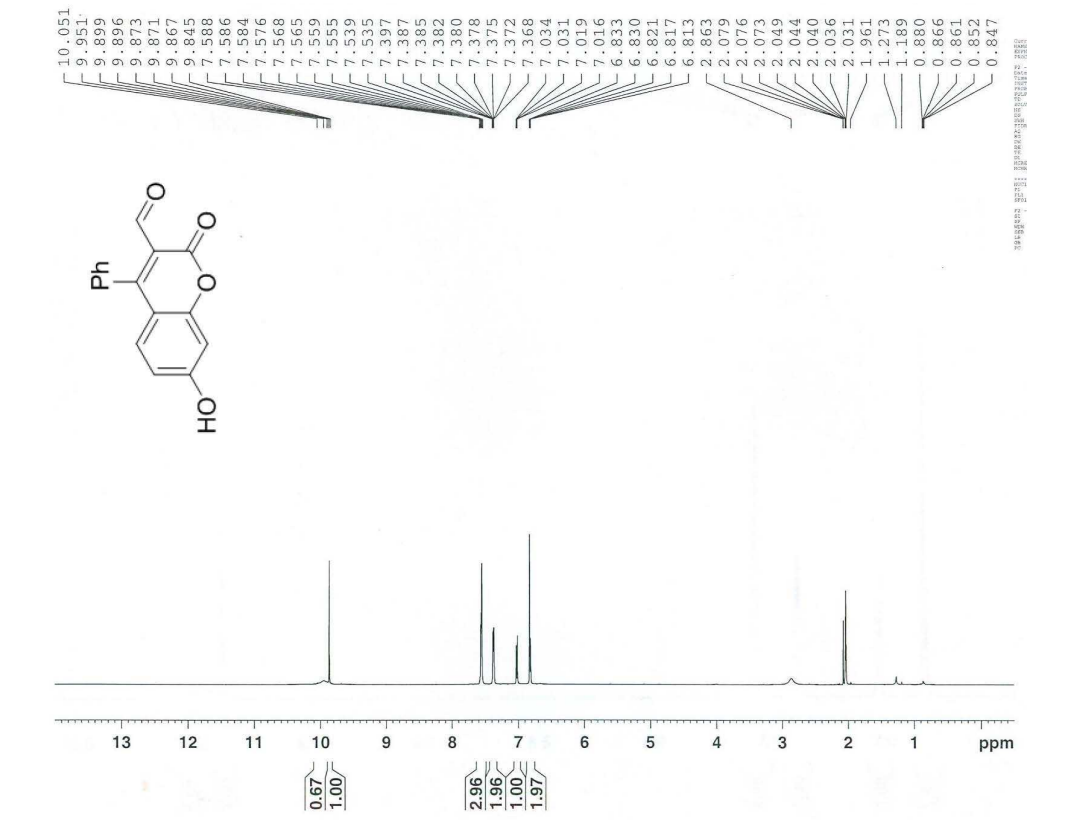
Compound 4. Compound **3** (465 mg, 1.746 mmol) and N-phenyltriflimide (686 mg, 1.921 mmol) were combined in a round bottom flask. THF (24 mL) was added and then DIPEA was added dropwise (0.38 mL, 2.270 mmol). The mixture stirred at ambient temperature for 3 h followed by removal of the solvent *in vacuo*. The residue was purified by chromatography (95:5 CH_2Cl_2/EtOAc) to yield compound **4** (607.2 mg, 87%) as a golden oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.95 (s, 1H), 7.56-7.62 (m, 3H), 7.33-7.37 (m, 2H), 7.29-7.32 (m, 2H), 7.16 (dd, 1H, *J* = 9.0, 2.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.5, 159.5, 157.1, 155.0, 152.6, 131.4, 130.8, 130.3, 129.0, 128.4, 119.9, 119.7 (q, C-F, *J* = 40 Hz), 118.0, 117.3, 110.6; IR (neat, cm^{-1}) 1765, 1605, 1552, 1422, 1364, 1217, 1136, 1107, 980; HRMS calculated for $C_{17}H_9F_3O_6SNa$ (M + Na^+): 420.9964. Found: 420.9961.



ES517. Compound **4** (123 mg, 0.309 mmol) was combined with N,N-dimethylsulfamide (42 mg, 0.340 mmol), Pd_2dba_3 (14 mg, 0.015 mmol), SPhos (18 mg, 0.046 mmol), and K_3PO_4 (132 mg, 0.618 mmol) in a round bottom flask and degassed for 20 min. Dry degassed THF was added and the mixture was purged with N_2 for 30 min followed by heating at 55 °C for 24 h. The solvent was evaporated with a stream of N_2, the crude product taken up in water (pH 5), and extracted with EtOAc (15 mL x 3). Purification by chromatography (95:5 CH_2Cl_2/EtOAc) gave **ES517** (54 mg, 47%) as a yellow solid (mp 195 C): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.91 (s, 1H), 7.52-7.58 (m, 3H), 7.27-7.31 (m, 2H), 7.19 (d, 1H, *J* = 2.0 Hz), 7.15 (d, 1H, *J* = 9.0 Hz), 6.93 (dd, 1H, *J* = 9.0, 2.0 Hz), 2.93 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 188.2, 161.5, 158.4, 155.9, 144.1, 131.6, 130.8, 129.9, 128.8, 128.4, 116.9, 115.1, 114.5, 104.8, 38.1; IR (KBr, cm^{-1}) 3260, 1732, 1610, 1528, 1377, 1140; HRMS calculated for $C_{18}H_{16}N_2O_5SNa$ (M + Na^+): 395.0672. Found: 395.0670.

III. NMR Spectra

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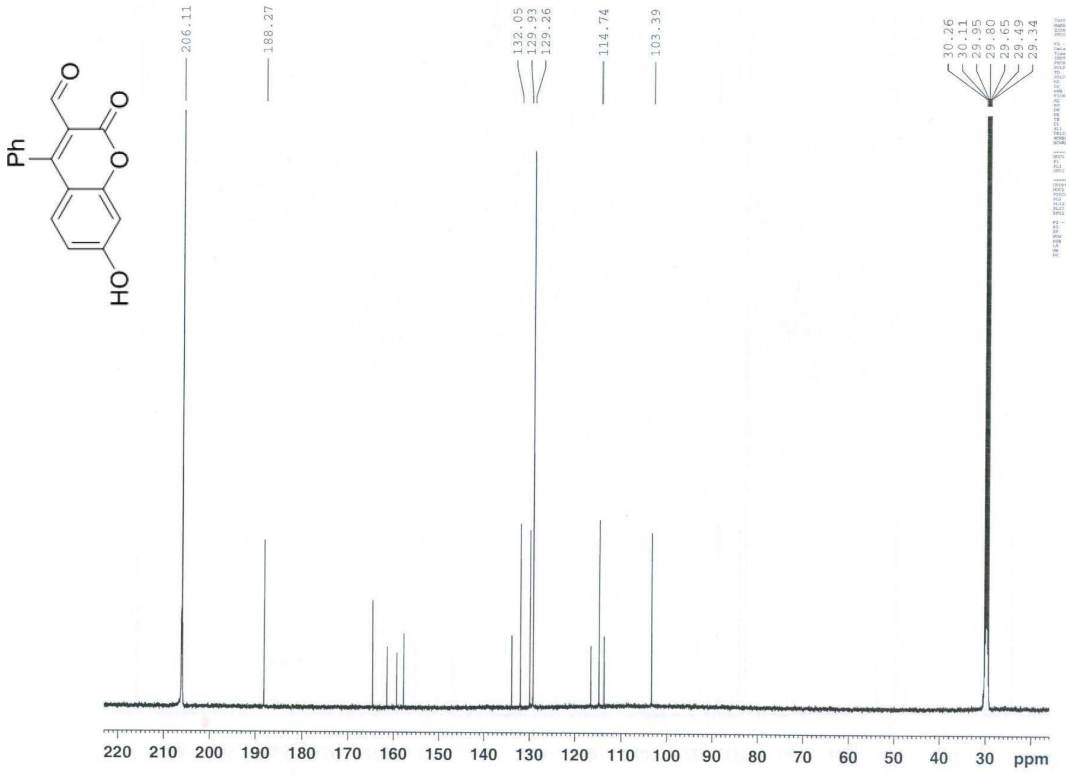
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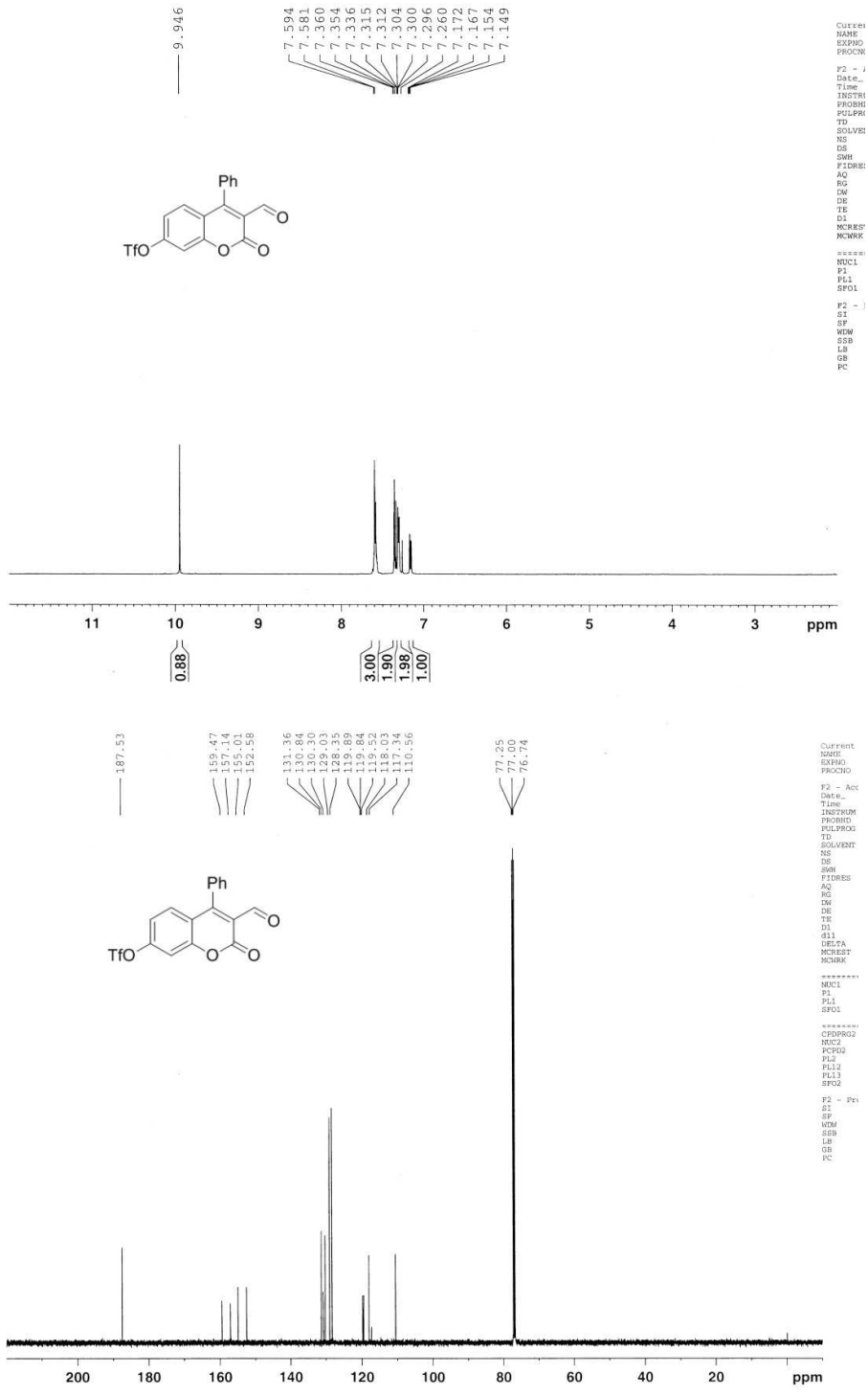
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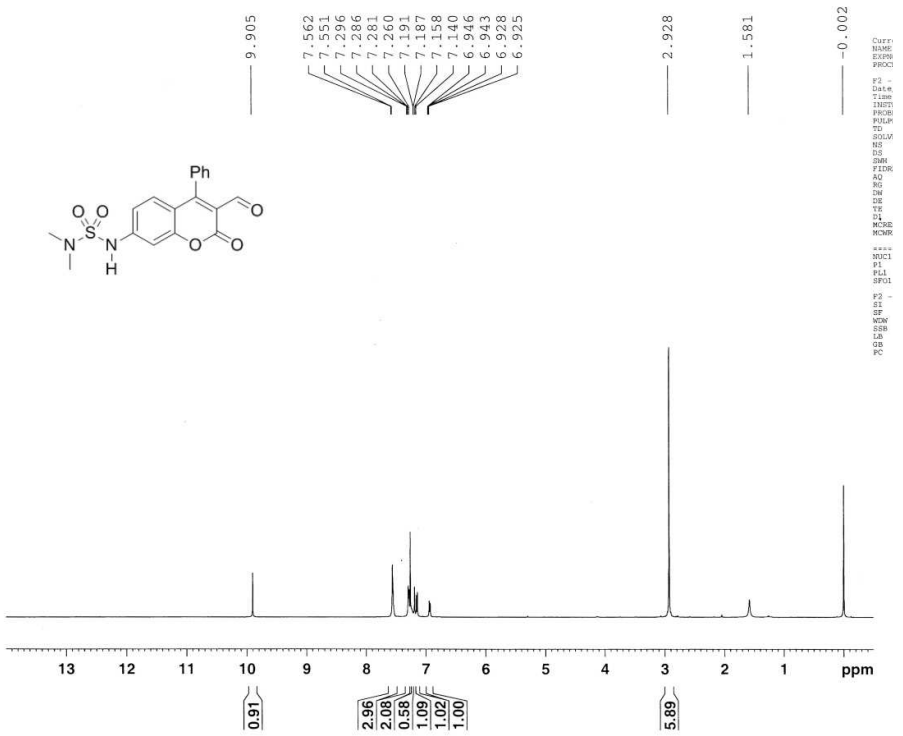
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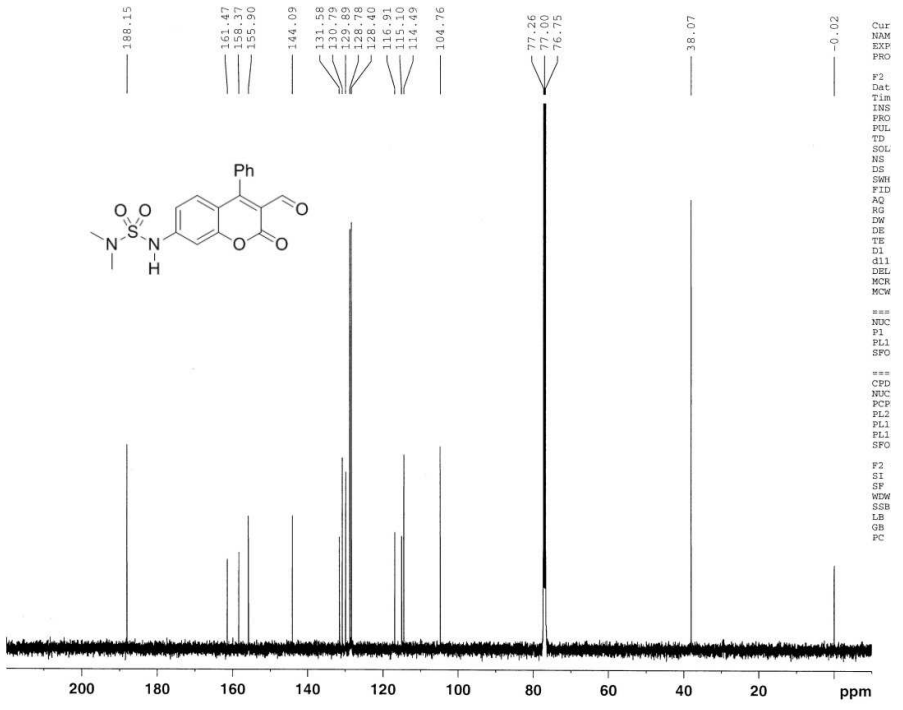
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